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(71) Applicant: ABBOTT LABORATORIES
14th Street and Sheridan Road North St
North Chicago Illinois 60064(US)

(72) Inventor: Heiman, Daniel Feulner
407 Drake Street
Libertyville Illinois 60048(US)

(74) Representative: Modiano, Guido et al,
MODIANO, JOSIF, PISANTY & STAUB Modiano & Associati Via Meravigli, 16
I-20123 Milan(IT)

(54) **Tracers for use in flecainide fluorescence polarization immunoassay.**

(57) The present invention is directed to a fluorescence polarization assay for determining the amount of flecainide in fluids, especially biological fluids such as serum, plasma or urine. The present invention also relates to novel tracer compounds useful in fluorescence polarization assay techniques.

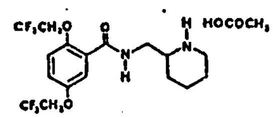

FIGURE 1

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TRACERS FOR USE IN FLECAINIDE FLUORESCENCE POLARIZATION IMMUNOASSAY

BACKGROUND OF THE INVENTION

Technical Field

The present invention relates to a method and reagents for a fluorescence polarization immunoassay (FPIA) procedure for determining the amount of flecainide in fluids, especially biological fluids such as serum, plasma or urine, and to a method for making certain of the reagents. More specifically, the invention relates to (1) reagents for determining the amount of flecainide in a sample, viz., tracer and buffer solutions; (2) synthetic methods for preparing the tracer compounds; and (3) analytical methods for conducting the assay.

Background Art

Flecainide acetate, or N-(2-piperidylmethyl)-2,5-bis (2,2,2-trifluoroethoxy)benzamide acetate, is a drug used to treat ventricular arrhythmias and tachycardia. Although flecainide acetate has been proven to be effective clinically, it can cause undesirable side effects if optimal dosage levels are exceeded. Therapeutic blood flecainide levels have been found to range from 0.2 to 1.0 microgram per milliliter, with 0.5 microgram per milliliter considered the mean effective concentration. Monitoring of serum flecainide levels combined with clinical data can provide the physician with useful information to aid in adjusting patient dosage, achieving optimal therapeutic effects while avoiding useless subtherapeutic or harmful toxic dosage levels.

In the past, patient serum or plasma flecainide lev ls have typically been measured by high performance liquid chromatography (HPLC) or gas chromatography (GC). Although thes methods ar r asonably specific for detecting drug levels, they are not without drawbacks. They involve extensive sample preparation, time-consuming instrument set-up and a need for highly trained personnel.

In assays for other substances, competitive binding immunoassays have provided a more satisfactory alternative. Typically, competitive binding immunoassays are used for measuring ligands in a test sample. (For purposes of this disclosure, a "ligand" is a substance of biological interest to be determined quantitatively by a competitive binding immunoassay technique.) The ligands compete with a labeled reagent, or "ligand analog," or "tracer," for a limited number of receptor binding sites on antibodies specific to the ligand and ligand analog. The concentration of ligand in the sample determines the amount of ligand analog which binds to the antibody: the amount of ligand analog that will bind is inversely proportional to the concentration of ligand in the sample, because the ligand and the ligand analog each bind to the antibody in proportion to their respective concentrations.

Fluorescence polarization provides a quantative means for measuring the amount of tracer-antibody conjugate produced in a competitive binding immunoassay. Fluorescence polarization techniques are based on the principle that a fluorescent labeled compound, when excited by plane polarized light, will emit fluorescence having a degree of polarization inversely related to its rate of rotation. Accordingly, when a tracer-antibody conjugate having a fluorescent label is excited with plane polarized light, the emitted

light remains highly polarized because the fluorophore is constrained from rotating between the time that light is absorbed and emitted. In contrast, when an unbound tracer is excited by plane polarized light, its rotation is much faster than that f the corresponding tracer-antibody conjugate. As a result, the light emitted from the unbound tracer molecules is depolarized.

Fluorescence polarization immunoassay techniques are well known in the art, to date, however, the use of such techniques for the determination of flecainide levels have not been successfully attempted. Thus, the present invention offers an advance in the art in that highly sensitive tracers, a method for making the tracers, and an assay using the tracers are provided specifically for the determination of flecainide. The assay conducted in accordance with the present invention is particularly accurate, as will be explained infra.

SUMMARY OF THE INVENTION

The present invention is directed to an FPIA for flecainide; to tracers for use in the assay; and to methods for making such tracers.

A first aspect of the invention relates to the discovery of unique tracers having novel structures. According to this first aspect of the invention, the tracers and their precursors can both be represented by the structural formula shown in Figure 2 of the drawings, wherein:

Q is hydrogen, hydroxyl or a leaving group, or fluorescein or a fluorescein derivative;

Z is NH, C=O, SO_2 or C=NH; and

R is a linking group including up to 10 heteroatoms and having a total of from 0 to 20 carbon atoms and heteroatoms arranged in a straight or branched chain and containing up to two ring structures.

When Q is fluorescein or a derivative thereof, the compound can be used as a tracer. When Q is hydrogen, hydroxyl or a leaving group, the structure represents a pr cursor used in pr paring a tracer. For purposes of this disclosure, a "leaving group" is a halogen, an acyloxy group (including a carbonate ester), a succinimidyloxy or phthalimidyloxy group, an alkoxy or phenoxy or substituted phenoxy group, an imidazolyl group, a benzotriazolyloxy group or any of the other similar activating groups well known to those skilled in the art.

According to a second aspect of the invention, a method is provided for making a tracer by coupling a compound represented by the structural formula shown in Figure 2, wherein:

Q is hydrogen, hydroxyl or a leaving group;

Z is NH, S, O, C=O, SO_2 or C=NH; and

R is a linking group including up to 10 heteroatoms, having a total of from 0 to 20 carbon atoms and heteroatoms arranged in a straight or branched chain and containing up to two ring structures;

with fluorescein or a derivative of fluorescein.

According to a third aspect of the invention, a process for measuring concentration of flecainide is provided. A sample is contacted with flecainide antiserum, and a fluorescein-containing flecainide derivative capable of producing a detectable fluorescence polarization response to the presence of the flecainide antiserum. Plane-polarized light is then passed through the solution to obtain a fluorescence polarization response, and this response is detected as a measure of the amount of flecainide in the sample.

Further objects and attendant advantages of the invention will be best understood from a reading of the following detailed description taken together with the Figures and the Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following Figures, the symbol "Fl" represents a fluorescein moiety, and the various other symbols are noted in the Detailed Description.

Figure 1 shows the general structure of the drug flecainide acetate to be quantitatively determined in accordance with the present invention.

Figure 2 shows a general structural formula for the tracers of the present invention, as well as the classes of reactants used in preparing them.

Figure 3 shows the alternate structural formulae and names of the fluorescein moiety included in the tracers of the present invention.

Figure 4 shows various linkages that couple the fluorescein moiety to the precursor in Figure 2, when Figure 2 represents a precursor for the tracers.

Figures 5 through 24 show various examples of structures of tracers in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The various aspects of the invention will now be discussed in detail in relation to the Figures.

Definitions and Explanatory Material

The present invention involves the use of fluorescein and derivatives of fluorescein. In particular, a necessary property of fluorescein and its derivatives for the usefulness of the tracer compounds is the fluorescence of fluorescein. Fluorescein exists in two tautomeric forms, illustrated in Figure 3, depending on the acid concentration (pH) of the environment. In the open (acid) form, there are a number of conjugated double bonds which make that form

of fluorescein (and compounds containing a fluorescein moiety) capable of absorbing blue light and emitting green fluoresc nc after an excited state lifetime of about four nanoseconds. When the open and closed forms coexist, the relative concentration of molecules in the open and closed forms is easily altered by adjustment of the pH level. Generally, the tracer compounds of the present invention exist in solution as biologically acceptable salts such as sodium, potassium, ammonium and the like, allowing the compounds to exist in their open, fluorescent form when employed in the analytical methods of the present invention. The specific salt present will depend on the buffer employed to adjust the pH level. For example, in the presence of a sodium phosphate buffer, the compounds in the present invention will generally exist in the open form, as a sodium salt.

As used herein, the term "fluorescein", either as an individual compound or as a component of a larger compound, is meant to include both the open and closed forms, if they exist for a particular molecule, except in the context of fluorescence. An open form is necessary for the fluorescence to occur.

The numbering of carbon atoms of the fluorescein molecule varies, depending upon whether the open or closed form of the molecule is considered. Accordingly, the literature concerning fluorescein and its compounds is not uniform as to carbon atom numbering. In the closed form, the para-carbon to the carbonyl of the lactone on the phenyl ring is numbered 6. In the open form, the para-carbon to the carboxylic acid group on the phenyl ring is numbered 5 (see Figure 3). In this disclosure the numbering of the closed form is adopted because the raw materials used in the syntheses are most popularly numbered with that system. The carbon atom of fluorescein and its compounds which

is opposite the carboxyl group is ther fore numbered "6" for the purposes of th present disclosure.

A tracer in solution which is not complexed to an antibody is free to rotate in less than the time required for absorption and re-emission of fluorescent light. As a result, the re-emitted light is relatively randomly oriented so that the fluorescence polarization of a tracer not complexed to an antibody is low, approaching zero. Upon complexing with a specific antibody, the tracer-antibody complex thus formed assumes the rotation of the antibody molecule, which is slower than that of the relatively small tracer molecule, thereby increasing the polarization observed. Therefore, when a ligand competes with the tracer for antibody sites, the observed polarization of fluorescence of the resulting mixture of the free tracer and tracer-antibody complex assumes a value intermediate between that of the tracer and that of the tracer-antibody complex. If a sample contains a high concentration of the ligand, the observed polarization value is closer to that of the free ligand, i.e., low. If the test sample contains a low concentration of the ligand, the polarization value is closer to that of the bound ligand, i.e., high. By sequentially exciting the reaction mixture of an immunoassay with vertically and then horizontally polarized light and analyzing only the vertically polarized component of the emitted light, the polarization of fluorescence in the reaction mixture may be accurately determined. The precise relationship between polarization and concentration of the ligand to be determined may conveniently be established by measuring the polarization values of calibrators with known concentrations. The concentration of the ligand can be interpolated from a standard curve prepared in this manner.

The particular tracers formed in accordance with this invention have b n found to pr duce very good assays, as discuss d infra.

The Reagents

The objective in designing a competitive binding immunoassay for flecainide is to set up a reasonably balanced competition between the drug flecainide and the tracer for the recognition sites on the antibody. Widely varying structures for haptens and tracers may be successful in achieving this goal. For the purposes of this invention, "haptens" are precursors of the immunogens, comprising generally a substituted flecainide derivative bearing a group suitable for linking to an immunologically active carrier.

The Antibodies

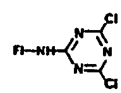
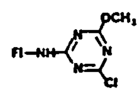
The antibodies employed in the present invention are prepared by eliciting a response in rabbits or sheep to appropriate immunogens. The immunogen is administered to animals or to in vitro cultures of immunocompetent cells by a series of innoculations, in a manner well known to those skilled in the art. It should be understood that although sheep were the preferred immune host to flecainide immunogens in the experiments detailed herein, any in vivo or in vitro host capable of producing antibodies may be employed.

The Structure of the Tracers

The tracers of the present invention can be represented by the general structural formula shown in Figure 2. In a preferred form of the invention, the tracer has the structural formula shown in Figure 5.

The tracer is a flecainide derivative that is linked to a fluorescein derivative by, for example, an amido, amidino, triazinylamino, carbamido, thiocarbamido, carbamoyl, sulfonamido, or sulfonylcarbamoyl group, as show in Figure 4. The tracers are prepared by linking the appropriate fluorescein derivative to a flecainide derivative containing an amino, carboxylic acid, sulfonic acid, hydroxy, imidate, hydrazide, isocyanate, chloroformate, chlorosulfonylcarbamoyl, or like group, as will be discussed in he context of the synthetic method and the Examples below.

By way of example, any of the following fluorescein derivatives can be used:

Fl-NH_2	fluorescein amine
Fl-CO_2H	carboxyfluorescein
Fl-$NHCOCH_2I$	α-iodacetamidofluorescein
Fl-$NHCOCH_2Br$	α-bromoacetamidofluorescein
	2,4-dichloro-1,3,5-triazin-2-ylamino-fluorescein (DTAF)
	4-chloro-6-methoxy-1,3,5-triazin-2-ylamino-fluorescein

The Synthesis of the Tracers

The tracers of the present invention are made by coupling a fluorescein moiety or a derivative of fluorescein, to the general structure shown in Figure 2 wherein Q is hydrogen, hydroxyl or a leaving group; Z is NH, S, O, C=O, SO_2 or C=NH, and R is a linking group including up to 10 heteroatoms and having a total of from 0 to 20 carbon and heteroatoms; arranged as a

straight or branched chain and containing up to two ring structures. Th fluorescein moiety can be linked to the amino, carboxyl, imidate or alkoxy functional group by an amide, an amidine, a urea, a carbamate, a sulfonamide, a triazinylamino or a sulfonylcarbamate linkage, as shown in Figure 4. In the presently preferred embodiment, the fluorescein derivative is dichlorotriazinylaminofluorescein (DTAF) (Isomer I), available from Research Organics, Cleveland, Ohio, and this is coupled to a precursor shown in Figure 2 (wherein $R=(CH_2)_3$, Z=NH and Q=H).

The DTAF is coupled to the aminoalkyl derivative of flecainide by stirring the two compounds in methanol between a temperature of about 0°C and the boiling point of the solvent. Basic compounds such as triethylamine may be added if desired, and especially if a salt (such as the hydrochloride or acetate) of the amine precursor is employed in the reaction. Other solvents, such as dimethylformamide or dimethylsulfoxide, may be used. Usable tracers can be prepared from a variety of flecainide derivatives.

All flecainide derivatives that have a terminal amino group, such as amino, hydrazinyl, hydrazido or the like, are coupled to carboxyfluorescein by the active ester method or the mixed anhydride method and coupled to the DTAF or alkoxy chlorotriazinylaminofluorescein by simply mixing the two materials in solution. The amino group can be converted to the isocyanate group by reaction with phosgene. This is then condensed with aminofluorescein to produce the tracer.

Flecainide derivatives having a terminal mercapto group are coupled in an inert solvent with α-bromoacetamidofluorescein or α-iodoacetamido-fluorescein.

All flecainide derivatives that have a terminal carboxylic acid group, such as carboxylic acid, (aminohydroxy)alkylcarboxylic acid or the like, are coupled to aminofluorescein by the active ester method or mixed anhydride methods.

All flecainide derivatives that have a terminal hydroxy group can be coupled to fluorescein by reaction with DTAF, α-iodoacetamidofluorescein or α-bromoacetamidofluorescein in solution. The hydroxy group can be converted to the chlorosulfonylcarbamoyl or chloroformate groups by reaction with chlorosulfonylisocyanate or phosgene, respectively. These derivatives are then coupled to aminofluorescein in solution to produce the tracer.

Sulfonic acid derivatives of flecainide are converted to the corresponding sulfonyl chlorides by reaction with a chlorinating reagent such as thionyl chloride, phosphoryl chloride, phosphorus pentachloride, or the like. The sulfonyl halides are then reacted with aminofluoresceins or other fluorescein derivatives bearing reactive amino groups, usually in the presence of an added basic compound, to give the tracers.

Flecainide derivatives that have a terminal nitrile group are converted to imidates in anhydrous alcohol in the presence of hydrogen chloride gas. The imidate is then coupled to fluorescein amine in solution to prepare the tracer.

The Assay

The particular tracers of the present invention have been found to measure levels of flecainide accurately and specifically in serum and plasma. Figure 1 shows the structure of the drug flecainide that can be quantitatively determined in accordance with the present invention. The assay of the present invention provides

a more rapid and convenient flecainide assay method than prior art methods, because it requires no specimen treatment before analysis, th reagents are chemically and thermally stable, the assay syst m has minimal cross-reactivity to flecainide-like compounds and, because of its simplicity, may be carried out rapidly on highly automated equipment.

In accordance with the analytical methods of the present invention, i.e., the methods of determining flecainide by a fluorescence immunoassay procedure using the tracer compounds of the invention, a sample containing or suspected of containing flecainide is intermixed with a biologically acceptable salt of a tracer and an antibody specific to flecainide and the tracer. The flecainide and tracer compete for a limited number of antibody sites, resulting in the formation of complexes. Because the concentration of tracer and antibody is maintained constant, the ratio of flecainide antibody complex to tracer-antibody complex formed is directly proportional to the amount of flecainide in the sample. Therefore, upon exciting the mixture with linearly polarized light and measuring the polarization of the fluorescence emitted by a tracer and a tracer-antibody complex, one is able to determine quantitatively the amount of flecainide in the sample.

The results can be quantified in terms of net millipolarization units, span (in millipolarization units) and relative intensity. The measurement of millipolarization units indicates the maximum polarization when a maximum amount of the tracer is bound to the antibody in the absence of any flecainide. The higher the net millipolarization units, the better the binding of the tracer to the antibody. The span is an indication of the difference between the net millipolarization at the points of maximum and minimum

amount of tracer bound to the antibody. A larger span provid s f r a better numerical analysis of data. The intensity is a m asure f the str ngth f the signal abov background. Thus, a high r intensity is d termined at about 0.5 to 2.0 nanomolar for the preferred tracers of the invention, as the sum of the vertically polarized intensity plus twice the horizontally polarized intensity. The intensity of the tracer signal can range from about three times to about thirty times the background noise, depending upon the concentration of the tracer and other assay variables. For the purposes of the present invention, an intensity of at least five times that of background noise is preferred.

Table I shows the results obtained with various embodiments of the present invention, in terms of span, millipolarization units and intensity. In all instances, the antiserum employed was raised in sheep. As can be seen from this data, an assay produced by use of the tracer of Figure 5 provides excellent results and is presently the most preferred. In addition, the tracers represented by Figures 16 and 21 also produced acceptable results and thus are alternative preferred tracers.

TABLE I

Tracer	Net Polarization*	Span*	Intensity**
Fig. 5	217	110	5.9
Fig. 6	240	60	4.5
Fig. 7	322	77	6.4
Fig. 8	189	39	8.1
Fig. 9	198	49	7.7
Fig. 10	220	86	9.1
Fig. 11	240	86	13.0
Fig. 12	217	83	7.8
Fig. 13	303	84	6.2
Fig. 14	223	62	5.5
Fig. 15	233	89	10.0
Fig. 16	289	104	19.0
Fig. 17	199	69	10.4
Fig. 18	212	65	7.0
Fig. 19	274	72	2.94
Fig. 20	285	79	4.8
Fig. 21	273	104	7.8
Fig. 22	216	69	11.6
Fig. 23	214	74	6.8

* In millipolarization units
** Expressed as the ratio of net intensity to background noise.

The pH at which the method of the present invention is practiced must be suffici nt to allow the fluoresc in moiety of th tracers to xist in their open f rm. Th pH may rang from about 3 to 12, more pr ferably in the range of from about 5 to 10, and most desirably from about 6 to 9. Various buffers may be used to achieve and maintain the pH during the assay procedure. Representative buffers include borate, citrate, acetate, phosphate, carbonate, tris, barbital and the like. The particular buffer employed is not critical to the present invention, but the tris and phosphate buffers are preferred. The cation portion of the buffer will generally determine the cation portion of the tracer salt in solution.

The preferred method of the improved assay of the present invention will now be discussed in detail The assay is a "homogeneous assay", which means that the end polarization readings are taken from a solution in which bound tracer is not separated from unbound tracer. This represents a distinct advantage over heterogeneous immunoassay procedures wherein the bound tracer must be separated from the unbound tracer before a reading can be taken.

The reagents for the fluorescence polarization assay of the present invention comprise antibody specific for flecainide and tracer. Additionally, largely conventional solutions including a flecainide pretreatment solution, a dilution buffer, flecainide calibrators and flecainide controls are desirably prepared. Typical solutions of these reagents, some of which are described herein, are commercially available in assay "kits" from Abbott Laboratories, Abbott Park, Illinois.

All percentages expressed herein are weight/volume unless otherwise indicated. The trac r

formulation presently preferred is 82 nanomolar tracer in: 0.1 molar tris buffer at pH 5.5; 5% 5-sulfosalicylate; and 0.1% sodium azide. The antiserum formulation comprises sheep antiserum diluted with: 1% normal sheep serum (volume/volume); and 0.1% sodium azide. The dilution buffer comprises: 0.1 molar sodium phosphate at pH 7.5; 0.1% sodium azide; and 0.01% bovine gamma globulin. The pretreatment formulation comprises: 0.1 molar tris buffer at pH 5.5; 5% 5-sulfosalicylate; and 0.1% sodium azide. Calibrators comprising flecainide in normal human serum at concentrations of 0.0, 0.1, 0.25, 0.50, 1.0 and 1.5 milligrams per liter, with 0.1% sodium azide preservative are useful. Controls comprising flecainide in normal human serum are provided at concentrations of 0.15, 0.60 and 1.20 milligrams per liter with 0.1% sodium azide as a preservative.

The preferred procedure is especially designed to be used in conjunction with the Abbott TDx® polarization analyzer available from Abbott Laboratories, Irving, Texas. 50 microliters of serum or plasma are required. The calibrators, controls, or unknown samples are pipetted directly into the sample well of the TDx® sample cartridge. One of the advantages of this procedure is that the sample does not require any special preparation. If a TDx® flecainide assay kit is being used with the TDx® analyzer, samples are placed directly into a sample carousel, the caps from each of the three reagent containers in the kit are removed and placed into designated wells inside the TDx® analyzer, and the assay procedure from this point is fully automated.

If a manual assay is being performed, the sample is mixed with the pretreatment solution in dilution buffer and a background reading is taken. The

tracer is then mixed with th assay. The antibody is then finally mixed into the t st solution. After incubation, a fluorescence polarization reading is taken.

The fluorescence polarization value of each calibrator, control or sample is determined and may be printed on the output tape of an instrument such as the Abbott TDx® polarization analyzer. A standard curve is generated in the instrument by plotting the polarization of each calibrator versus its concentration using nonlinear regression analysis. The concentration of each control or sample is read off the stored calibration curve and printed on the output tape.

With respect to the foregoing preferred procedure, it should be noted that the tracer, antibody, pretreatment solution, calibrators and controls should be stored between about 2 and about 8°C, while the dilution buffer should be stored at ambient temperature. A standard curve and controls should be run every two weeks, with each calibrator and control run in duplicate. Controls should be run daily and all samples can be run in replicates if so desired.

It should be understood that the foregoing Detailed Description and the following Examples are intended to be illustrative, but not limiting, with respect to the scope of the present invention. Various modifications will become apparent to one skilled in the art, and thus it is intended that the scope of the invention be defined solely by the claims and legal equivalents thereof.

EXAMPLES

Examples 1 through 19 describe experiments that were performed in accordance with the concepts of the present invention.

Example 1

Pr paration of the Tracer of Figure 6

Flecainide ac tate (9.5 mg) and 2-(fluorescein-5-yl)amino-4,6-dichloro-1,3,5-triazine (8.5 mg) were dissolved in 0.15 ml of methanol and stirred at ambient temperature for 3 1/2 hours. The reaction mixture was streaked directly onto a thin-layer silica gel plate and chromatographed with chloroform/methanol to give the tracer.

Example 2

Preparation of the Tracer of Figure 7

Flecainide acetate (9.5 mg) and 2-(fluorescein-6-yl)amino-4,6-dichloro-1,3,5-triazine (8.0 mg) were taken up on 0.15 ml of methanol and stirred at ambient temperature for 16 hours. The reaction mixture was streaked onto a thin-layer silica gel plate and chromatographed with chloroform/methanol to give the tracer.

Example 3

Preparation of the Tracer of Figure 8

Flecainide acetate (23.7 mg), 5-bromoacetamidofluorescein (15.6 mg) and triethylamine (0.0116 ml) were dissolved in 0.25 ml of methanol and stirred at ambient temperature for 48 hours. The product was isolated by chromatography on a thin layer silica gel plate developed with chloroform-methanol.

Example 4

Preparation of the Tracer of Figure 9

Flecainide hemisuccinamide (25.7 mg), triethylamine (0.0066 ml) and isobutyl chloroformate

(6.8 mg) were dissolved in 0.4 ml of dimethylformamide chilled in an ice bath and stirred for 1 hour. The solution of activated precursor was divided in two, 5-aminofluorescein (8.7 mg) was added to one portion, the mixture was allowed to warm to ambient temperature and stirring was continued for 14 hours. The solvent was removed and the residue was chromatographed on thin layer silica gel plates with chloroform-methanol and again with benzene-ethyl acetate-acetone to give the pure tracer.

Example 5

Preparation of the Tracer of Figure 10

One half of the solution of activated precursor prepared in Example 4 was stirred at ambient temperature with 8.7 mg of 6-aminofluorescein for 15 hours. The dimethylformamide was removed in vacuo, and the residue was chromatographed on thin layer silica gel plates with chloroform-methanol and again with benzene-ethyl acetate-acetone to give the pure tracer.

Example 6

Preparation of the Tracer of Figure 11

The precursor of Figure 24 (0.02 millimoles) and 6-(N-succinimidyloxycarbonyl)fluorescein (11.8 mg) were stirred in 0.35 ml of dimethylformamide for 4 hours at ambient temperature. The solvent was removed, and the residue was chromatographed on thin layer silica gel plates with chloroform-methanol and again with benzene-ethyl acetate-acetone to give the pure tracer.

Example 7

Preparation of the Tracer of Figure 12

This compound was prepared according to the method of Example 6 from 0.02 millimoles of the

precursor of Figure 25 and 11.8 mg of 6-(N-succinimidyloxycarbonyl)fluorescein, chromatographing first with benzene-ethyl acetate-hexane and then with chloroform-methanol.

Example 8

Preparation of the Tracer of Figure 13

The precursor of Figure 25 (0.02 millimoles) and 2-(fluorescein-6-ylamino)-4,6-dichloro-1,3,5-triazine were taken up in 0.40 ml of methanol and stirred at ambient temperature overnight. The product was purified by chromatography on thin layer silica gel developed with chloroform-methanol and with benzene-ethyl acetate-acetone.

Example 9

Preparation of the Tracer of Figure 14

The compound was prepared according to the method of Example 8 from 0.02 millimoles of the precursor of Figure 26 and 13.3 mg of 2-(fluorescein-5-ylamino)-4,6-dichloro-1,3,5-triazine in 0.20 ml of methanol. Chromatographic purification was with chloroform-methanol.

Example 10

Preparation of the Tracer of Figure 15

This compound was prepared according to the method of Example 6 from 0.02 millimoles of the precursor of Figure 26 and 11.8 mg of 6-(N-succinimidyloxycarbonyl)fluorescein in 0.25 ml of dimethylformamide.

Example 11

Preparation of the Tracer of Figure 16

This compound was prepared according to the method of Example 8 from 0.02 millimoles of the precursor of Figure 27 and 13.3 mg of 2-(fluorescein-6-

ylamino)-4,6-dichloro-1,3,5-triazine in 0.20 ml of methanol.

Example 12

Preparation of the Tracer of Figure 17

This compound was prepared according to the method of Example 6 from 0.02 millimoles of the precursor of Figure 27 and 11.8 mg of 6-(N-succinimidyl-oxycarbonyl)fluorescein in 0.25 ml of dimethylformamide. Chromatography was with chloroform-methanol.

Example 13

Preparation of the Tracer of Figure 18

This compound was prepared according to the method of Example 6 from 0.02 millimoles of the precursor of Figure 28 and 11.8 mg of 6-(N-succinimidyl-oxycarbonyl)fluorescein in 0.20 ml of dimethylformamide. Chromatography was with chloroform-methanol.

Example 14

Preparation of the Tracer of Figure 19

A mixture of 0.02 millimoles of the precursor of Figure 27 and 13.2 mg of 2-(fluorescein-5-ylamino)-4-methoxy-6-chloro-1,3,5-triazine in 0.50 ml of methanol was stirred and heated in a 50° oil bath for 6 1/2 hours. The crude reaction mixture was streaked onto a thin-layer silica gel plate and developed with chloroform-methanol to give the pure tracer.

Example 15

Preparation of the Tracer of Figure 20

This compound was prepared according to the method of Example 14 from 0.02 millimoles of the

precursor of Figure 27 and 13.2 mg of 2-(fluorescein-6-ylamino)-4-methoxy-6-chloro-1,3,5-triazine. Chromatography was with chloroform-methanol and benzene- thyl ac tat -acetone.

Example 16

Preparation of the Tracer of Figure 21

A mixture of N-(3-aminopropyl)flecainide (Figure 29) and 5.3 mg of 2-(fluorescein-6-ylamino)-4,6-dichloro-1,3,5-triazine was stirred at ambient temperature in 0.2 ml of methanol for 24 hours. The product was purified by chromatography on silica gel thin layer plates with chloroform-methanol and benzene-ethyl acetate-acetone.

Example 17

Preparation of the Tracer of Figure 5

This compound was prepared according to the method of Example 16, except that the 5-isomer instead of the 6-isomer of the fluorescent labeling reagent was employed.

Example 18

Preparation of the Tracer of Figure 22

The precursor of Figure 29 (4.7 mg) and 6-(N-succinimidyloxycarbonyl)fluorescein (4.7 mg) were stirred in 0.20 ml of dimethylformamide for 24 hours at ambient temperature. The solvent was removed, and the residue was chromatographed on a thin layer silica gel plate with chloroform-methanol, followed by a second chromatography on a C_{18}-reversed-phase plate developed with water-methanol containing 0.5% acetic acid.

Example 19

Preparation of the Tracer of Figure 23

This compound was prepared by the method of Example 18, except that the 5-isomer of the activated

carboxyfluorescein was employed instead of the 6-isomer. Purification was by chromatography twic on silica gel plat s with chloroform-methanol.

CHARACTERIZATION OF FLECAINIDE TRACERS

Tracer of Figure 5

Ultraviolet absorbance spectrum: Lambda max= 494 nm
Mass spectrum (FAB technique): (M+H)+ = 930 amu
NMR spectrum (300 MHz in CD3OD): delta 1.4 to 1.8 ppm, m, 8H; 2.3, m, 1H; 2.6, m, 2H; 3.05, m, 4H; 4.4 to 4.7, m, 4H; 6.6, m, 4H; 7.05 to 7.2, m, 6H; 7.35, m, 1H; 7.45, d, 1H; 7.9, t, 1H.

Tracer of Figure 10

Ultraviolet absorbance spectrum: Lambda max = 494 nm.

Tracer of Figure 11

Ultraviolet absorbance spectrum: Lambda max = 498.7 nm

Tracer of Figure 15

Ultraviolet absorbance spectrum: Lambda max = 499.7 nm

Tracer of Figure 16

Ultraviolet absorbance spectrum: Lambda max = 495.5 nm

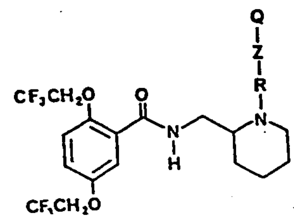
Tracer of Figure 21

Mass spectrum (FAB technique): (M+H)+ = 930 amu, (M+Na)+ = 952 amu, (M+2Na)+ = 974 amu.

NMR spectrum (300 MHz in CD3OD: delta 1.4 ppm, m, 4H; 1.65, m, 4H, 2.05, m, 1H; 2.25, m, 2H; 2.7, m, 2H; 3.05, m, 4H; 4.4 to 4.6, m, 4H; 6.55, m, 4H;6.55,m,4H; 7.05 to 7.15, m, 6H; 7.4, d, 1H;

CLAIMS:

1. A compound comprising th structure:



wherein:

Z is a linking group including up to 10 heteroatoms, and having a total of from 0 to 20 carbon atoms and heteroatoms arranged in a straight or branched chain and containing up to two ring structures;

A is NH, C=O, SO_2 or C=NH; and

Q is fluorescein or a fluorescein derivative.

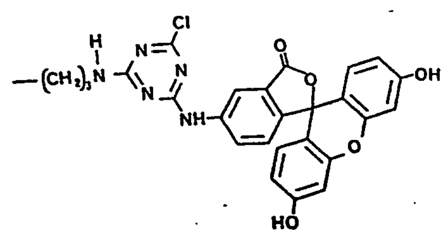
2. The compound of Claim 1 wherein Q is (fluorescein-5-yl)amino.

3. The compound of Claim 1 wherein Q is (fluorescein-6-yl)amino.

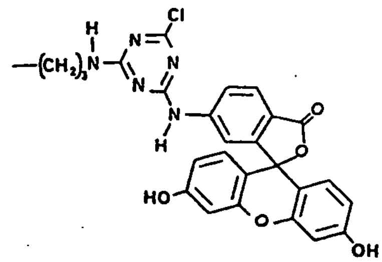
4. The compound of Claim 1 wherein Q is (fluorescein-5-yl)carbonyl.

5. The compound of Claim 1 wherein Q is (fluorescein-6-yl)carbonyl.

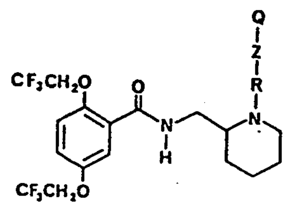
6. The compound of Claim 1 wherein -R-Z-Q has the following structure:



7. The compound of Claim 1 wherein -R-Z-Q has the following structure:



8. A method for making a tracer comprising the step of coupling a precursor of the formula



wherein:

R is a linking group including up to 10 heteroatoms and having a total of from 0 to 20 carbon atoms and heteroatoms arranged in a straight or branched chain and containing up to two ring structures;

Z is NH, S, O, C=O, SO_2 or C=NH; and

Q is hydrogen, hydroxyl or a leaving group;

with fluorescein or a derivative of fluorescein.

9. A process for measuring the concentration of flecainid in an aqueous s lution which comprises the steps of:

a) contacting a sample with a flecainide antiserum and with a compound according to Claim 1 capable of producing detectable fluorescence polarization response to the presence of flecainide antiserum;

b) passing plane polarized light through the solution resulting from step a) to obtain a fluorescence polarization response; and

c) detecting the fluorescence polarization response of the solution of step b) as a measure of the amount of flecainide in the sample.

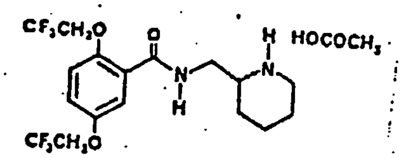


FIGURE 1

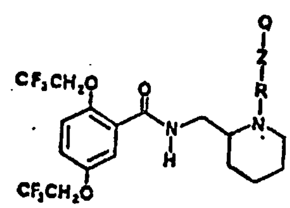


FIGURE 2

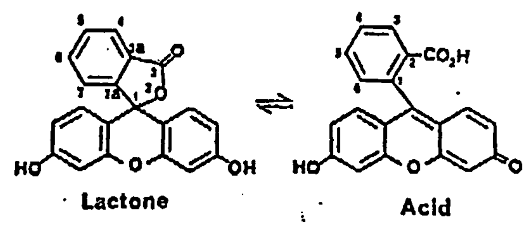


FIGURE 3

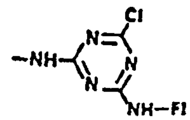


Fig. 4-1

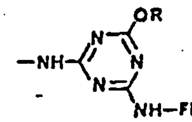


Fig. 4-2

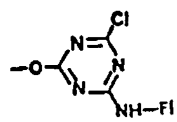


Fig. 4-3

-NH-CO-Fl

Fig. 4-4

-CO-NH-Fl

Fig. 4-5

-C(NH)-NH-Fl

Fig. 4-6

-NH-CO-NH-Fl

Fig. 4-7

-NH-CS-NH-Fl

Fig. 4-8

-O-CO-NH-Fl

Fig. 4-9

-O-CS-NH-Fl

Fig. 4-10

-SO_2-NH-Fl

Fig. 4-11

-O-CO-NH-SO_2-NHFl

Fig. 4-12

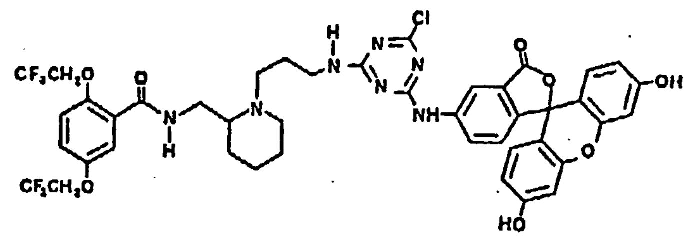


FIGURE 5

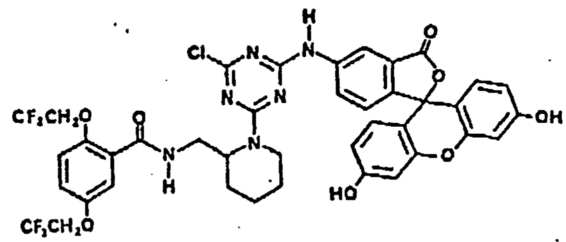


FIGURE 6

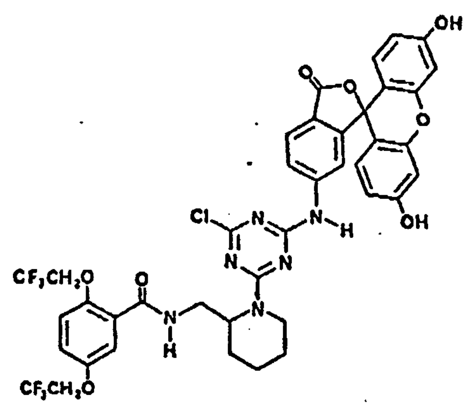


FIGURE 7

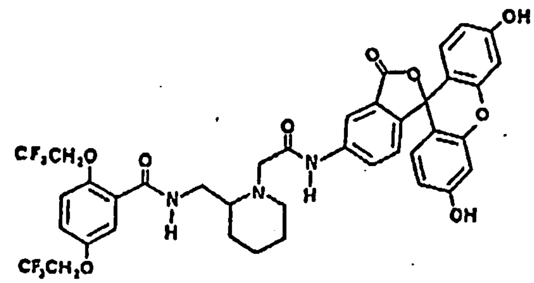


FIGURE 8

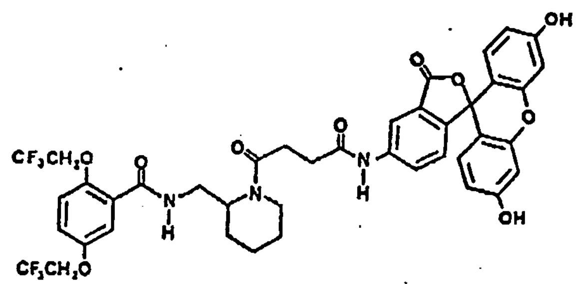


FIGURE 9

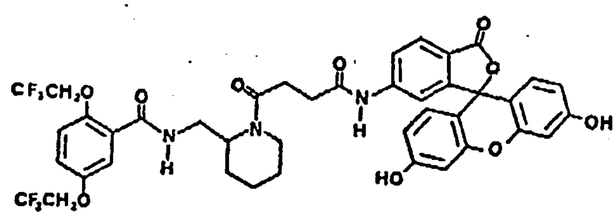


FIGURE 10

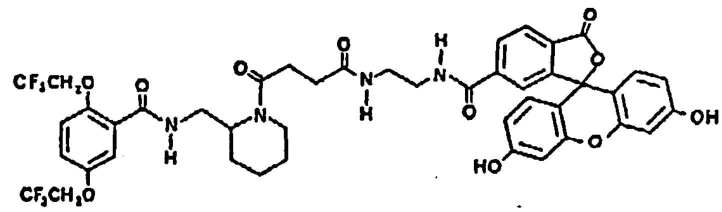


FIGURE 11

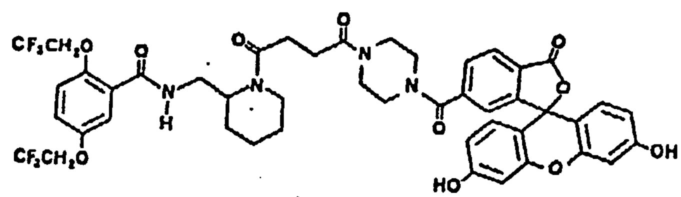


FIGURE 12

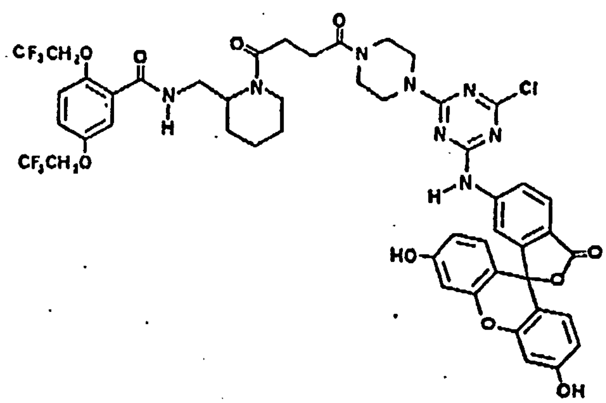


FIGURE 13

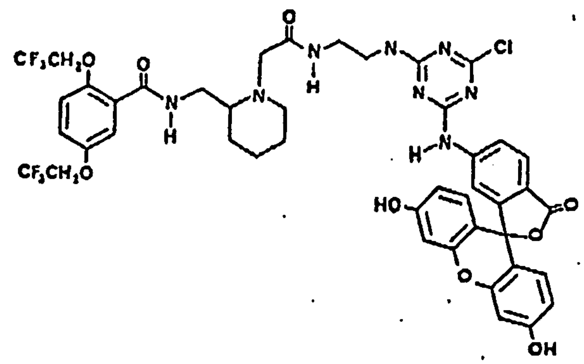


FIGURE 14

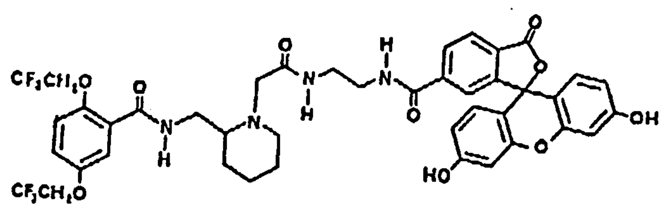


FIGURE 15

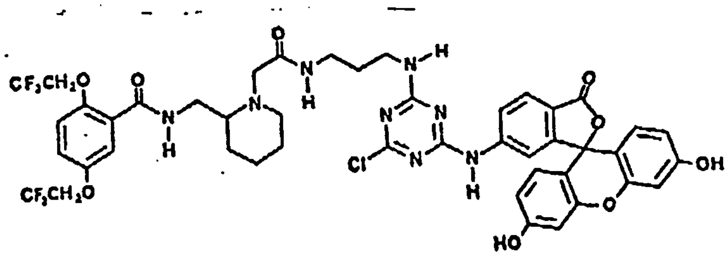


FIGURE 16

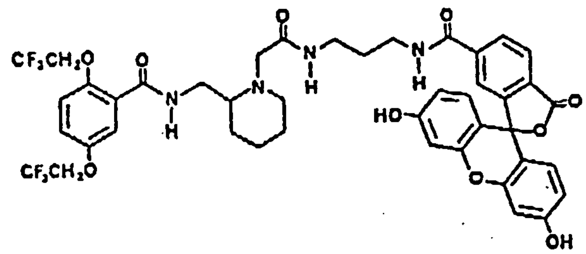


FIGURE 17

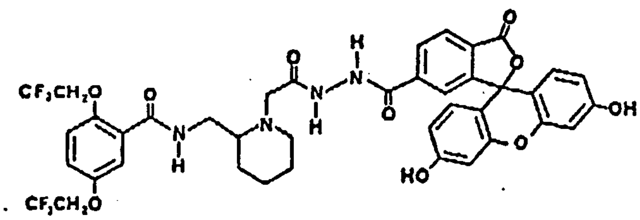


FIGURE 18

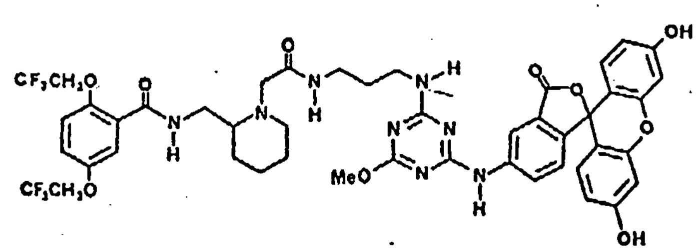


FIGURE 19

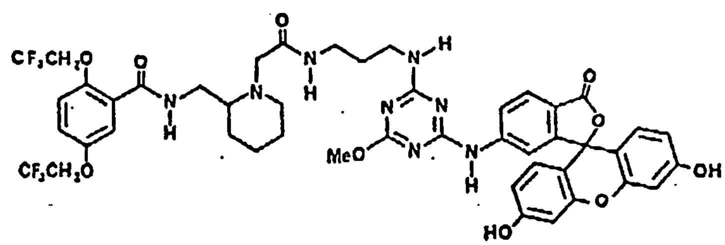


FIGURE 20

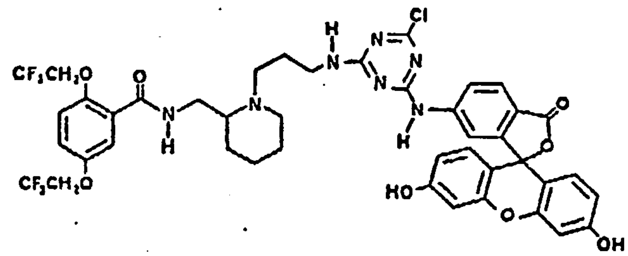


FIGURE 21

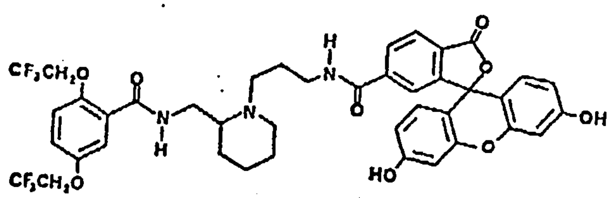


FIGURE 22

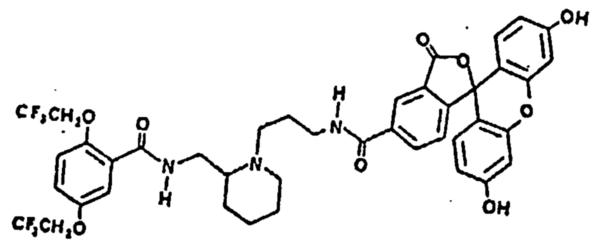


FIGURE 23